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Sep. 30, 2004

US 2004/0191233 A1

★ is a division of application Serial No. 09/884,894, Filed June 19, 2001 (pending), which

BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY AND METHODS OF USE

CONTINUING APPLICATION DATA

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 60/212,273, filed Jun. 19, 2000, which is incorporated by reference herein.

BACKGROUND

[0002] Microbes have been used extensively as probiotics. The generally accepted definition for a probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, *J. Appl. Bacteriol.*, 66, 365-378 (1989)). The host animals targeted to date by commercially available probiotics include farm animals (including chickens, pigs, calves), pets (including dogs), and humans. While probiotics for farm animals have been commercially available since the 1960's, their market impact has been hampered by the use of antibiotics in animal feed and lack of knowledge on the probiotic mechanism of action (Fuller, in: *Probiotics: A Critical Review*, Tannock (ed.), Horizon Scientific Press, Norfolk, UK, pp. 15-2 (1999)). However, a resurgence of research interest in the probiotic field (and controversy surrounding antibiotics in animal feed) is now directing renewed interest in the animal probiotic market. The human probiotic market is also of vital importance to agriculture in the U.S. and other countries because of the association of human probiotics and dairy products. This association occurs because these microbes often survive best in dairy products and probiotics are presently delivered to consumers in the U.S. via milks and yogurts. The potential to use fruit juice sweetened dairy drinks for probiotic delivery, especially to children, is evident from the success of this approach in Europe and Asia. While the human probiotic market is quite significant in Europe and Asia, particularly Japan, it has only recently received attention by the U.S. food industry (Sanders, *Food Technol.*, 53, 67-77 (1999)). The growing U.S. interest in this market is primarily because of the increasing number of health-conscious consumers in the U.S. and the potential health benefits which are attributed to probiotics. The potential benefits include: increased resistance to gastrointestinal tract infections; alleviation of constipation; reestablishment of a healthy intestinal flora following antibiotic or chemotherapy treatments; stimulation of the immune system; reduction of serum cholesterol; prophylactic for intestinal cancers; and alleviation of the symptoms of lactose intolerance (reviewed in O'Sullivan et al., *Int. Dairy J.*, 8, 513-525 (1998); Sanders, *Food Technol.*, 53, 67-77 (1999)).

[0003] One type of probiotic is Bifidobacterium. Microbes representing the genus Bifidobacterium were first described by Tissier over one hundred years ago when studying the predominant microbes in the stools of breast fed infants. Tissier initially referred to these microbes as Bacillus and it was not until 1924 that the genus Bifidobacterium was proposed to classify these unique microbes. Their taxonomic position, however, remained in doubt throughout most of this century as many researchers thought they belonged in the genus Lactobacillus, primarily because of morphological and fermentative similarities (reviewed, Poupard et al., *Bacteriol. Rev.*, 37, 136-165 (1973)).

[0004] Morphologically, bifidobacteria are rods of various shapes and often arranged in star-like or "V" patterns,

typically called 'bifid' arrangements. They are nonmotile, nonspore forming and are strictly anaerobic (Scardovi, V. *Bergey's Manual of Systematic Bacteriology*, vol. 2., Sneath et al., (ed.) The Williams & Wilkins Co., Baltimore, MD, pp. 1418-1434 (1986)). The primary habitats of these microbes are the intestines of humans and many animals. Consequently, they are also found in sewage and, as a result, have been proposed as indicators of fecal contamination (Resnick et al., *Appl. Environ. Microbiol.*, 42, 433-438 (1981)). Their presence in the human intestine, primarily the large intestine, is almost universally accepted to be a contributing factor to a healthy well-being.

[0005] Production of Bacteriocins by Lactic Acid Bacteria, including Bifidobacteria

[0006] The ability of lactic acid bacteria (LAB) to act as a preservative has been exploited in numerous dairy products for thousands of years. Their major preservative effect is due to the production of organic acids, primarily lactic acid. However, it is also known that many of them produce antimicrobial proteins, which have inhibitory properties against other related bacteria (Nes et al., *Antonie van Leeuwenhoek*, 70, 113-128, (1996)). All of the LAB bacteriocins characterized to date only inhibit certain Gram positive bacteria and exhibit no native activity against any Gram negative microbes. The range of activity against Gram positive bacteria varies with the bacteriocin. Some have a broad spectrum of activity such as nisin, which is produced by *Lactococcus lactis*, and has activity against most Gram positive bacteria (Hansen, *Crit. Rev. Food Sci. Nutr.*, 34, 69-93 (1994)). Others, such as Caseicin 80, which is produced by *Lactobacillus casei*, have activity only against strains of *L. casei* and possibly other closely related species (Rammelsberg and Radler, *J. Appl. Bacteriol.*, 69, 177-184 (1990)).

[0007] There have been a variety of reports indicating that bifidobacteria exhibit antibacterial action and this is primarily due to the production of lactic and acetic acids, which can inhibit the proliferation of pathogenic bacteria. Studies have indicated that bifidobacteria are effective at inhibiting the growth of *E. coli* (Ibrahim and Bezkorovainy, *J. Food Prot.*, 56, 713-715 (1993); Sasaki et al., *J. Vet. Med. Sci.*, 56, 433-437 (1994)), members of the genera *Salmonella* (Oyarzabal and Conner, *Poul. Sci.*, 74, 1418-1425 (1995); Gibson and Wang, *J. Appl. Bacteriol.*, 77, 412-420 (1994)), *Listeria*, *Campylobacter*, *Shigella* as well as *C. perfringens* and *Vibrio cholerae* (Gibson and Wang, *J. Appl. Bacteriol.*, 77, 412-420 (1994)). It has been suggested that bifidobacteria may be able to produce broad spectrum anti-microbial inhibitors, other than organic acids (Gibson and Wang, *J. Appl. Bacteriol.*, 77, 412-420 (1994)). However, there is not yet any convincing data published on the presence of any broad spectrum anti-microbial compound from bifidobacteria, other than organic acids. The only available evidence for a proteinaceous antimicrobial compound produced by a Bifidobacterium strain is the bacteriocin Bifidocin B from *B. bifidum* NCFB 1454, which is only active against certain Gram positive bacteria (Yildirim and Johnson, *J. Food Prot.*, 61, 47-51 (1998)).

[0008] Importance of Iron

[0009] With the exception of certain lactobacilli, all known microbes require iron for growth (Archibald, *FEMS Microbiol. Letts.*, 19, 29-32 (1983)). Because of the extreme

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PATENT
Docket No. 110.01290102IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | | |
|---------------|---------------|---|-----------------|---------|
| Applicant(s): | O'SULLIVAN |) | Group Art Unit: | Unknown |
| | |) | | |
| Serial No.: | Unassigned |) | Examiner: | Unknown |
| (Parent:) | 09/884,894 |) | | |
| | |) | | |
| Filed: | Herewith |) | | |
| (Parent:) | June 19, 2001 |) | | |

For: BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY AND
METHODS OF USE

PRELIMINARY AMENDMENT

Commissioner for Patents
Mail Stop PATENT APPLICATION
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

The present application is a divisional patent application of Serial No. 09/884,894, filed June 19, 2001, which claims the benefit of U.S. Provisional Application Serial No. 60/212,273, filed June 19, 2000.

Prior to taking up the above-identified application for examination, please amend the application as follows:

Amendments to the Specification begin on the page entitled "Amendments to the Specification."

Amendments to the Claims are reflected in the listing of claims which begins on the page entitled "Amendments to the Claims."

Remarks begin on the page entitled "Remarks."

Preliminary Amendment

Applicant(s): O'SULLIVAN

Serial No. Unknown (Parent Serial No. 09/884,894)

Filed: Herewith (Parent: June 19, 2001)

For: BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY AND METHODS OF USE

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Page 2 of 6

Amendments to the Specification

Please replace the paragraph beginning at page 1, line 8, with the following amended paragraph.

This [[application]] is a division of application Serial No. 09/884,894, filed June 19, 2001 (pending), which claims the benefit of U.S. Provisional Application Serial No. 60/212,273, filed June 19, 2000, which [[is]] are all incorporated by reference herein.

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Receipt is hereby acknowledged for the following in the U.S. Patent and Trademark Office:

Applicant(s): O'SULLIVAN
Filed: Herewith
Title: BIFIDOBACTERIA AND SIDEROPHORES PRODUCED
THEREBY AND METHODS OF USE

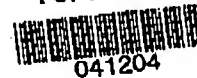
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Enclosed: Specification (29 consecutively numbered pgs, including 58 claims on 6 consecutively numbered pgs and a 1 pg. Abstract); copy of Declaration (3 pgs); Application Data Sheet (2 pgs); Request for Filing a Divisional Patent Application Under Rule 1.53(b) (3 pgs); Preliminary Amendment (6 pgs); check for \$385.00 for the filing fee; and transmittal document (in triplicate).

Mailed: April 12, 2004
Docket: 110.01290102

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Patent
Docket No. 110.01290102BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY
AND METHODS OF USE

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CONTINUING APPLICATION DATA

This application claims the benefit of U.S. Provisional Application Serial No. 60/212,273, filed June 19, 2000, which is incorporated by reference herein.

10

BACKGROUND

Microbes have been used extensively as probiotics. The generally accepted definition for a probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, *J. Appl. Bacteriol.*, 66, 365-378 (1989)). The host animals targeted to date by commercially available probiotics include farm animals (including chickens, pigs, calves), pets (including dogs), and humans. While probiotics for farm animals have been commercially available since the 1960's, their market impact has been hampered by the use of antibiotics in animal feed and lack of knowledge on the probiotic mechanism of action (Fuller, In: *Probiotics: A Critical Review*, Tannock (ed), Horizon Scientific Press, Norfolk, UK. pp. 15 - 2 (1999)). However, a resurgence of research interest in the probiotic field (and controversy surrounding antibiotics in animal feed) is now directing renewed interest in the animal probiotic market. The human probiotic market is also of vital importance to agriculture in the U.S. and other countries because of the association of human probiotics and dairy products. This association occurs because these microbes often survive best in dairy products and probiotics are presently delivered to consumers in the U.S. via milks and yogurts. The potential to use fruit juice sweetened dairy drinks for probiotic delivery, especially to children, is evident from the success of this approach in Europe and Asia. While the human probiotic market is quite significant in Europe and Asia, particularly Japan, it has only recently received attention by the U.S. food industry (Sanders, *Food Technol.*, 53, 67-77 (1999)). The growing U.S. interest in this market is primarily because of the increasing number of health-conscious